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Abstract

Ferrous chloride solution was injected unilaterally into the sensorimotor cortex of rats to induce a chronic epileptic focus. Accumulation of cyclic AMP elicited by depolarizing agents was determined in slices from different cortical areas of rats 30-60 days after the injection. In anterior cortical areas which include the sensorimotor cortex, the cyclic AMP accumulation elicited by ouabain or a high concentration of potassium ion was greater in electrographic spike activity on the dominant side than on the other. In posterior cortical areas, no difference in cyclic AMP accumulation was detected. The regional difference in the depolarization-elicited accumulation of cyclic AMP is discussed with regard to the process of epileptic focus.

KEYWORDS: cyclic AMP, depolarization, cortical slices, ferrous chloride, focal epilepsy, rat

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—— BRIEF NOTE ——

DEPOLARIZATION-ELICITED ACCUMULATION OF CYCLIC AMP IN SLICES OF RAT CEREBRAL CORTEX WITH A CHRONIC EPILEPTIC FOCUS

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Abstract. Ferrous chloride solution was injected unilaterally into the sensorimotor cortex of rats to induce a chronic epileptic focus. Accumulation of cyclic AMP elicited by depolarizing agents was determined in slices from different cortical areas of rats 30-60 days after the injection. In anterior cortical areas which include the sensorimotor cortex, the cyclic AMP accumulation elicited by ouabain or a high concentration of potassium ion was greater in electrographic spike activity on the dominant side than on the other. In posterior cortical areas, no difference in cyclic AMP accumulation was detected. The regional difference in the depolarization-elicited accumulation of cyclic AMP is discussed with regard to the process of epileptic focus.

Key words : cyclic AMP, depolarization, cortical slices, ferrous chloride, focal epilepsy, rat.

Fluctuations in cyclic nucleotide levels in the brain have been reported in relation to experimental models of epilepsy (1). These reports have sparked attempts to investigate the response of the cyclic AMP-generating system to neurotransmitters and neuromodulators in cortical slices from epileptic rats. The results have indicated that accumulation of cyclic AMP elicited by adenosine (2, 3), norepinephrine (4) or glutamate (5), is different among areas of rat cerebral cortex showing epileptic discharges in electrocorticograms (ECoGs).

Besides these compounds, depolarizing agents, such as ouabain, veratridine and high concentrations of potassium ion, have also been demonstrated to elicit cyclic AMP accumulation in mammalian brain slices (6-8). The present study was undertaken to compare the cyclic AMP accumulation elicited by ouabain or a high concentration of potassium ion in slices from different areas of rat cerebral cortex, in which electrographic spike discharges were produced by unilateral injection of ferrous chloride solution.

Materials and Methods. Male Wistar rats weighing 210-270 g were used. Under ether anesthesia, 5 μl of $FeCl_2$ solution was injected into the left sensorimotor

cortex of rats in essentially the same manner as Willmore *et al.* (9). Rats used as controls received an injection of saline in the same way. Then, stainless steel electrodes were implanted in the cranial bone for ECoG recording. After the surgery, ECoGs were recorded at least once a week.

Rats were killed by decapitation 30-60 days after the injection. The cerebrum was extirpated and dissected into four parts : the left anterior, right anterior, left posterior, and right posterior quadrants. The injection site was in the left anterior quadrant. Cross-chopped cortical slices were prepared from each quadrant with a McIlwain tissue chopper. The cortical slices (2-4 mg protein) were preincubated for 30 min in 5 ml of Krebs-Ringer bicarbonate-glucose buffer. After the preincubation, the medium was replaced with 5 ml of fresh buffer alone or buffer containing 0.1 mM ouabain or 100 mM KCl. The increase in the KCl concentration was offset by an equivalent decrease in NaCl. The slices were further incubated for 10 min. The preincubation and incubation were performed at 37 ℃ with constant aeration with 95 % O_2-5 % CO_2. At the end of the incubation, the medium was discarded and 2.5 ml of cold 7 % trichloroacetic acid was added to the slices. After homogenization of the resultant mixture in an ice-bath, cyclic AMP contents were assayed using a cyclic AMP assay kit (Radiochemical Centre, Amersham, U.K.) based on the method of Gilman (10) following purification of cyclic AMP by column chromatography (11), as described previously (5). The protein content of the homogenate was determined by the method of Lowry *et al.* (12).

Results. In ECoGs of $FeCl_2$ solution-injected rats, isolated spikes were seen at frontal leads of both hemispheres. These spike discharges were accompanied with very little abnormal behavior. Cyclic AMP contents of incubated cortical slices of rats 30-60 days after injection of $FeCl_2$ solution or saline are shown in Table 1. In animals of the left dominant group, ECoG spike frequency on the left side was more than twofold that on the right, and in animals of the right dominant group, the spike frequency on the right was more than twofold that on the left. Cyclic AMP contents of slices after incubation in the buffer alone were not different between left and right cortical areas in both anterior and posterior halves of the cortex of $FeCl_2$ solution-injected animals. However, in animals of the same groups, the cyclic AMP contents were different among the cortical areas when the slices were incubated with 0.1 mM ouabain or 100 mM KCl.

As shown in Table 1, the cyclic AMP contents were elevated 4- to 6-fold by incubation with ouabain. In animals of the left dominant group, the cyclic AMP accumulation elicited by ouabain was greater in the left anterior area than in the right anterior area. In animals of right dominant group, the cyclic AMP accumulation was greater in the right anterior area than in the left anterior area. In the posterior areas, no difference was detected in animals of both the left and right dominant groups. Table 1 also shows that the cyclic AMP contents were elevated 4- to 5-fold by incubation in buffer containing 100 mM KCl and that

the cyclic AMP accumulation differed regionally as that after incubation with ouabain.

In animals of the saline-injected group, neither ECoG spike discharges nor abnormal behavior were observed. In these animals, there was no regional difference in the cyclic AMP contents of cortical slices after incubation under any condition (Table 1).

TABLE 1. CYCLIC AMP CONTENTS OF INCUBATED SLICES FROM FOUR CORTICAL AREAS

Group and cortical area	Cyclic AMP (pmol/mg protein)		
	No addition	Ouabain	Potassium ion
$FeCl_2$-injected			
Left dominant			
Left anterior	12.7 ± 0.9	79.6 ± 6.0[a]	63.1 ± 6.3[c]
Right anterior	11.5 ± 0.7	52.7 ± 4.4	44.6 ± 2.0
Left posterior	12.4 ± 1.0	57.3 ± 3.0	57.1 ± 2.6
Right posterior	11.9 ± 0.6	51.8 ± 2.2	52.8 ± 2.9
Right Dominant			
Left anterior	11.6 ± 0.6	56.4 ± 3.8	43.8 ± 2.5
Right anterior	12.4 ± 0.8	73.1 ± 4.0[b]	59.0 ± 4.8[c]
Left posterior	12.2 ± 0.8	55.9 ± 5.1	56.9 ± 4.6
Right posterior	11.1 ± 1.0	58.1 ± 6.0	56.2 ± 3.7
Saline-injected			
Left anterior	12.4 ± 0.8	57.4 ± 5.8	58.5 ± 4.9
Right anterior	12.1 ± 0.5	55.7 ± 3.3	57.0 ± 4.3
Left posterior	13.2 ± 0.5	62.8 ± 3.9	64.1 ± 5.9
Right posterior	13.7 ± 0.4	62.0 ± 4.0	63.7 ± 3.0

Cortical slices were prepared from rats 30-60 days after injection of $FeCl_2$ solution or saline. Concentrations of ouabain and KCl were 0.1 and 100 mM, respectively. Values are the mean ± SEM for 7-10 different experiments. [a, b, c]Significantly greater than the contralateral cortical area as determined by Student's *t*-test : *a*, $p < 0.005$; *b*, $p < 0.01$; *c*, $p < 0.02$.

Discussion. In anterior cortical areas of $FeCl_2$ solution-injected rats, cyclic AMP accumulation elicited by ouabain or a high concentration of potassium ion was greater in electrographic spike activity on the dominant side of the cortex than on the other. Studies, mostly using guinea pigs, on elicitation of cyclic AMP accumulation by depolarizing agents have revealed that the accumulation, at least in part, is mediated by extracellular adenosine (6-8). Recently, we reported that there is a regional difference in cyclic AMP accumulation in response to adenosine in slices from rat cerebral cortex with an iron-induced epileptic focus (2, 3). Alterations of the adenosine-sensitive cyclic AMP system, therefore, may contribute to regional differences in cyclic AMP accumulation although little is known of how cyclic AMP accumulation is elicited by depolarizing agents in rat

cerebral cortical slices.

Changes in Na^+, K^+-ATPase activity have been reported in relation to the process of epilepsy. In cortex with an ethyl chloride focus, Na^+, K^+-ATPase activity was increased (13, 14), while in cortex with a focus induced by cobalt (15) or alumina cream (14) the enzyme activity was decreased. Such changes in Na^+, K^+-ATPase activity may be related to altered depolarization-elicited accumulation of cyclic AMP in epileptic cortex. However, this possibility remains to be substantiated since it is unknown whether or not changes in the enzyme activity occur in the process of iron-induced epileptic focus.

In posterior cortical areas, unlike the anterior cortex, no difference in the cyclic AMP accumulation elicited by ouabain or a high concentration of potassium ion was detected. The results of the present study suggest that the regional difference in cyclic AMP accumulation elicited by depolarizing agents is related to the differences in the excitability level in cortex with a chronic iron-induced epileptic focus.

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